INSTRUCTION FOR USE

GeneProof®
Molecular diagnostics for your routine

GeneProof SARS-CoV-2 Advanced PCR Kit

CE IVD

1. LIST OF PRODUCT VARIANTS

Product	Package	REF
GeneProof SARS-CoV-2 Advanced PCR Kit	25 reactions	COV2A/GP/025
GeneProof SARS-CoV-2 Advanced PCR Kit	100 reactions	COV2A/GP/100

2. INTENDED PURPOSE AND USE

Indication	*In vitro* diagnostic medical device
Regulatory Status	CE IVD / EC Directive 98/79/EC
Function	Diagnostics of SARS-CoV-2 and aid to diagnosis of COVID-19
What is Detected / Target	SARS-CoV-2
Automated / Manual detection	Manual
Type of Analysis	Qualitative
Validated Specimen	Swab in transport medium (PBS, physiological saline solution, UTM) or in Bi-CoV® or in nuclease-free water (NFW)
Testing Population	EU population
Intended User	For professional use in laboratories with trained staff
Test Principle	Real-time polymerase chain reaction (PCR) – amplification of the specific Target Sequence and detection using TaqMan probes with fluorophore-based detection

3. TECHNICAL SPECIFICATION

Target Sequence	*RdRp, N* and *E* genes
Analytical Specificity	SARS-CoV-2

Analytical Sensitivity (LoD with 95% probability)

Sample Processing	Channel	Sensitivity	Material
GeneProof PathogenFree RNA Isolation Kit	FAM	691.08 IU/ml	PBS
	Cy5	127.24 IU/ml	
croBEE 201A Nucleic Acid Extraction Kit	FAM	1404.51 IU/ml	PBS
	Cy5	1654.57 IU/ml	
croBEE®max Nucleic Acid Extraction Kit	FAM	1127.56 IU/ml	PBS
	Cy5	1779.36 IU/ml	
Direct detection (Bi-CoV®)	Cy5	5943.21 IU/ml	Bi-CoV®

Diagnostic Specificity	100 % ($CI_{95\%}$: 99.16 % - 100.00 %)
Diagnostic Sensitivity	100 % ($CI_{95\%}$: 97.10 % - 100.00 %)
Positive Predictive Value	100 % ($CI_{95\%}$: 97.10 % - 100.00 %)
Negative Predictive Value	100 % ($CI_{95\%}$: 99.16 % - 100.00 %)
Reporting Units	IU/ml
Metrological Traceability	1st WHO International Standard for SARS-CoV-2 RNA (NIBSC code: 20/146)
Extraction / Inhibition Control	Proper sampling, RNA extraction efficiency, reverse transcription and PCR inhibition control by endogenous Internal control (*RNase P* gene)
Validated Extraction Methods	croBEE 201A Nucleic Acid Extraction Kit GeneProof PathogenFree RNA Isolation Kit croBEE®max Nucleic Acid Extraction Kit

Applied Instruments

Instrument Name	*RdRp*	*E/N*	*RNase P*
croBEE Real-Time PCR System	FAM	Cy5	HEX
AMPLilab Real-Time PCR System	FAM	Cy5	HEX
AriaMx Real-Time PCR System	FAM	Cy5	HEX
BioQuant-96, Fluorescent Quantitative Detection PCR system	FAM	Cy5	HEX
CFX96™/ Dx Real-Time PCR Detection System	FAM	Cy5	HEX
Gentier 96E/96R Real-time PCR system	FAM	Cy5	HEX
LightCycler® 480	FAM	Cy5	HEX
LineGene 9600 Plus	FAM	Cy5	HEX
Mic qPCR Cycler	FAM	Cy5	HEX
QuantStudio™ 5 Real-time PCR system	FAM	Cy5	VIC
Rotor-Gene 3000 / Q	FAM	Cy5	HEX
SLAN® Real-Time PCR System	FAM	Cy5	HEX

Detection Channels	FAM (*RdRp*), Cy5 (*E/N),* HEX/VIC (*RNase P*)
External Quality Assessment	Regularly tested in QCMD and INSTAND e.V. External Quality Assessment Panels - results at *www.geneproof.com*

4. INTERFERENCES

The evaluation and settings of pathological values for interference testing was performed according to CLSI guidelines EP7-A2.

Endogenous and Exogenous Interferences

Tested Substance	Tested Level(s)	Observed Interference	Tested Substance	Tested Level(s)	Observed Interference
SWAB					
Whole blood	2 % (v/v)	None	Xylometazoline	0.25 mg/ml	None
Mucin	60 µg/ml	None	Phenylephrine	0.375 mg/ml	None
Sodium chloride	6.5 mg/ml	None	Neomycin, bacitracin	330 I.U. (neomycin) 25 I.U. (bacitracin)	None
Oxymetazoline	0.0625 mg/ml	None	Budesonide	100 µg	None

Summary: The tested interferents were shown not to interfere with the GeneProof PCR Kit.

5. KIT CONTENT

Vial Title	Cap Colour	Guaranteed Volume [µl]	Number of Vials	
			COV2A/GP/025 – for 25 rxn	COV2A/GP/100 – for 100 rxn
Master Mix COV2A	Blue	375	1	4
Positive Control SARS CoV2 10^4 cp/µl	White	200	1	2

6. CALIBRATOR INFORMATION

No calibrators **–** qualitative detection only.

7. TRANSPORT AND STORAGE

Storage Conditions	(-20 ± 5) °C
Transport Conditions	-20 °C and below
In-use Stability	3 thawings or for a maximum of 30 days after the first use of a particular vial

8. ASSAY PROCEDURE

SPECIMEN COLLECTION, TRANSPORTATION AND HANDLING

- Samples must be collected and transported following professional guidelines, at temperature (2 – 8) °C.
- Samples must be transported and processed by the laboratory in the shortest possible time (preferably within 24 hours).

If a transport media is used (see chapter 2. Intended Purpose and Use), proceed to the NUCLEIC ACID PURIFICATION step.

DIRECT DETECTION (NFW)

- Add (1.5 – 3) ml of NFW to the plastic tube with a swab. The head of the swab needs to be fully submerged. Vortex the sample.
- Transfer 150 µl of the clinical sample into a new plastic tube.
- Incubate the new plastic tube in a dry heat block for 10 minutes at 90 °C (short spin on vortex after 5 minutes and continue with incubation).
- Centrifuge the sample for 1 minute at 11 000 g.
- Place the sample in a cooling rack for 10 minutes at (2 – 8) °C.
- Use the prepared sample (supernatant) directly for PCR detection.

DIRECT DETECTION (BI-COV®)

- Follow the Instruction for Use provided with the Bi-CoV® set.
- After sampling, vortex and briefly centrifuge the clinical material collected in the Bi-CoV® medium and continue the procedure directly to the PCR setup. The Internal control is included in the collected sample.

NOTE: Direct detection with NFW may perform lower sensitivity in comparison with the procedure requiring nucleic acid extraction.

NUCLEIC ACID PURIFICATION

- Prepare specimens for the assay according to the corresponding extraction kit manual.
- Continue extraction according to the appropriate protocol.

PCR SETUP PROTOCOL

- Thaw required vials and reagents completely.
- Gently vortex and briefly centrifuge all vials before setting up the PCR run.
  NOTE:
  Keep the reagents at (2 – 8) °C for the shortest time possible until the PCR reaction is set up.
- Add 15 µl of Master Mix into PCR tubes.
- Add 10 µl of the extracted nucleic acid sample or 10 µl of Positive Control into the individual PCR tubes and mix by pipetting. The total reaction mix volume is 25 µl.
- Close the tubes, centrifuge shortly, insert them into the real-time PCR device and amplify according to the following PCR profile.
  NOTE:
  It is recommended to perform at least 1 negative control and at least 1 positive control (for a qualitative kit) for each individual PCR run. Use your own negative control (not provided) in the form of nuclease-free water. For more information see chapter 10. Run Validity.

AMPLIFICATION PROFILE
Follow the thermocycler manufacturer's manual for setting the instrument and for analysis.

FAST PCR Profile

Step	Process	Temperature [°C]	Time	Cycles	Fluorescence Acquisition
1	UNG decontamination/ Reverse transcription	50	5 min	1 cycle	
2	Initial denaturation	95	2 min	1 cycle	
3	Denaturation	95	3 s	45 cycles	
	Annealing/Extension	60	30 s		FAM, Cy5, HEX/VIC

9. INTERPRETATION OF RESULTS

Channel FAM (*RdRp*)	Channel Cy5 (*E*, *N*)	Channel HEX/VIC (*RNase P*)	Result	Interpretation
+	+	+/-	Valid	SARS-CoV-2 positive
+	-	+/-	Valid	SARS-CoV-2 positive
-	+	+/-	Valid	SARS-CoV-2 positive
-	-	+	Valid	SARS-CoV-2 negative
-	-	-	Invalid	-

NOTE: For interpretation of PCR run see chapter 10. Run Validity.

10. RUN VALIDITY

OVERALL VALIDITY OF DETECTION

	Signal	Channel	Run Validity	Recommendation
Positive Control	+	FAM, Cy5	Valid	-
Positive Control	-	FAM, Cy5	Invalid	Repeat PCR run
Negative control	-	FAM, Cy5	Valid	-
Negative control	+	FAM, Cy5	Invalid	Repeat PCR run

NOTE: If the issue persists, please contact Customer Support, see Contact information.

11. QUANTITATIVE DETECTION EVALUATION

Qualitative detection only.

12. ADDITIONAL PRODUCTS

No additional products.

13. MATERIAL AND DEVICES REQUIRED BUT NOT PROVIDED

Consumable material
96-well reaction plates or PCR tubes (0.2 ml volume), pipette tips with filters, powder-free gloves, biohazard waste bin, nuclease-free water

Devices
Real-time PCR instrument (see chapter 3. Technical Specification), nucleic acid extraction system or kit (see chapter 3. Technical Specification), desktop centrifuge (for 0.2 ml PCR tubes or 96-well plates), vortex mixer, freezer (-20 ± 5) °C, refrigerator (5 ± 3) °C, dry heat block, cooling rack

14. WARNINGS, PRECAUSIONS AND PROCEDURE LIMITATIONS

- Read the whole Instruction for Use properly before starting the manipulation. Not following these instructions can lead to an erroneous result which can cause misdiagnosis or inappropriate treatment!
- Use all necessary protective equipment (protective disposable gloves, a laboratory coat and eye protection) when handling specimens and kit reagents.
- Avoid microbial and ribonuclease contamination of the reagents when removing aliquots from reagent vials.
- Use RNase- and DNase-free filter pipette tips only.
- Use new tips for each pipetting step.
- Use separate working places for sample preparation / nucleic acid extraction and amplification reactions. Never introduce an amplified product in reagent and/or nucleic acid extraction (sample preparation) area.
- Close the kit components vials immediately after use and never interchange lids.
- Do not pool reagents from different lots or from different vials within the same lot.
- Do not substitute the reagents between different lots.
- Do not use kit after the expiry date.
- Do not use reagents from damaged or leaking vials.
- Dispose of unused reagents and waste in accordance with national, federal, state or local regulations.
- Use only with validated specimens (see chapter 2. Intended Purpose and Use) otherwise incorrect results could occur.
- The presence of UNG decontamination step reduces the risk of lower levels of amplicon contamination. However, contamination from very high levels of amplicons can be avoided only by good laboratory practices and careful adherence to the procedures specified in this Instruction for Use.
- Be very careful when handling the Positive Control or the clinical material; incorrect handling could result in contamination and the consequent impairment of the kit components! The manufacturer is not responsible for the kit impairment due to incorrect handling.

- This product is designed for use with the applied PCR instruments and validated extraction methods mentioned in chapter 3. Technical Specification.

Limitations:

- Patient management decisions should never be made based solely on the results from this test. Other laboratory and clinical factors must also be considered in making clinical decisions.
- Any serious incident occurred in relation to the using of GeneProof PCR Kit shall be reported to the manufacturer and to the competent local authority.

15. EXPLANATION OF SYMBOLS

Symbol	Explanation	Symbol	Explanation
CE	this product complies with the relevant EU requirements	LOT	Lot number
IVD	*in vitro* diagnostic medical device	Σ	Contains sufficient amount for n-tests
REF	Catalogue number		Temperature limitation
	Manufacturer		Expiry date
	Read Instruction for Use		

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